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(U) Molecular Mechanisms of Chemosensory Receptors, Signal Transducers, and the Activation of Gene Expression Controlling Establishment of a Marine Symbiosis

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Morse, Daniel Edward

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The first year's progress is reported in our research analyzing the chemical signal receptors, signal tranducers, and mechanisms controlling specific gene expression, in the regulation of larval metamorphosis in the marine mollusc, Haliotis rufescens. We have partially purified and begun in vitro characterization of the larval chemosensory receptors and signal transducers controlling metamorphosis in response to exogenous chemical signals. We have cloned and begun sequence analysis of the insulin gene. Expression of this gene is activated early in metamorphosis; its peptide hormone product is an important regulator of early growth.

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July 1, 1988

ANNUAL REPORT ON CONTRACT N0014-87-K-0762; R&T CODE 4412027

PRINCIPAL INVESTIGATOR: Daniel E. Morse

CONTRACTOR: University of California, Santa Barbara, CA

CONTRACT TITLE: Molecular Mechanisms of Chemosensory Receptors, Signal Transducers, and the Activation of Gene Expression Controlling Establishment of a Marine Symbiosis

PROJECT PERIOD: August 1, 1987 - July 31, 1988

RESEARCH OBJECTIVE:

(1) To characterize the molecular mechanisms by which marine invertebrate larval chemosensory receptors and their associated signal transducers mediate the larval recognition of signal molecules (peptides) produced by specific marine algae and bacteria, and then regulate host-induced larval metamorphosis and establishment of the marine symbiosis between the animal and algae; and (2) To characterize the molecular mechanisms regulating the activation of specific gene expression in the developmentally arrested marine invertebrate larva, in response to peptide inducers of symbiotic association with algae and bacteria.

PROGRESS (Year 1):

Objective 1: Chemosensory receptors and signal transducers: We have succeeded in partially purifying and completing the first *in vitro* analyses of the chemosensory receptors and signal transducers of the two pathways (the Morphogenetic Pathway, and the Regulatory Pathway; see Figure) that control the metamorphosis of *Haliotis* (marine mollusc) larvae in response to exogenous chemical signals. The partially purified sensory receptors have been shown to retain normal binding of both the morphogenetic peptide (and related GABA analogs bound by the Morphogenetic receptor), and of the faciliting diamino acids (bound by the Regulatory receptor), as confirmed by Scatchard analyses of radioligand binding *in vitro*. Down-regulation of the larvae by precocious exposure to morphogenetic GABA analogs prior to purification results in a marked reduction in the number of

Morphogenic receptors detectable *in vitro*, as predicted from results obtained *in vivo*, thus confirming the identification of the partially purified receptors as those that specifically control larval metamorphosis.

We are presently analyzing the detailed molecular mechanisms of the morphogenesis-regulating chemosensory signal transduction, *in vitro*. Thus far we have verified that the signal transduction pathway controlled by the Regulatory (i.e., diamino acid) receptor remains intact *in vitro*; specific ligand binding to this receptor activates a membrane-associated G protein, confirming the identification of this protein as the signal transducer in our earlier experiments performed *in vivo*. We are presently determining whether this G protein activates a specific protein kinase C in the *in vitro* preparation, as it does *in vivo*.

Objective 2: Mechanisms of morphogenetic activation of specific gene expression in the developmentally arrested larva: We have succeeded recently in cloning the gene coding for the insulin or insulin-like growth factor (IGF) from *Haliotis*. Expression of this gene is activated early in the morphogenesis of the larva, and its peptide hormone product has been shown to be an important regulator of early post-metamorphic growth.

Molecular hybridization under reduced stringency conditions permitted detection (in Northern blots) of a unique molluscan poly $A^+$-mRNA containing sequences homologous to those in known insulin and IFG genes. The tissue-specific distribution of this 0.75 kb mRNA parallels the tissue-specific distribution of the insulin peptide which we detect by radioimmune assay. Full-length cDNA was made from this mRNA; the resulting cDNA was tailed with EcoR1 linkers, purified on Sepharose CL-48, and cloned into a lambda-lac phage expression vector. The cloned inserted sequence was then detected by hybridization in Southern analyses, amplified, excised by EcoR1 digestion, purified by agarose gel electrophoresis, and finally subcloned (in both orientations, to facilitate sequence analysis) in a modified M13 vector.

Sequence analysis of the cloned gene is now in progress, using Sanger's double-stranded M13 dideoxy method, with the recent improvement of high resolution through the use $^{35}S$-labelled nucleotide. Thus far, approximately 50% of the sequence of the gene has been determined, and the probable reading-frame of the gene has been identified.

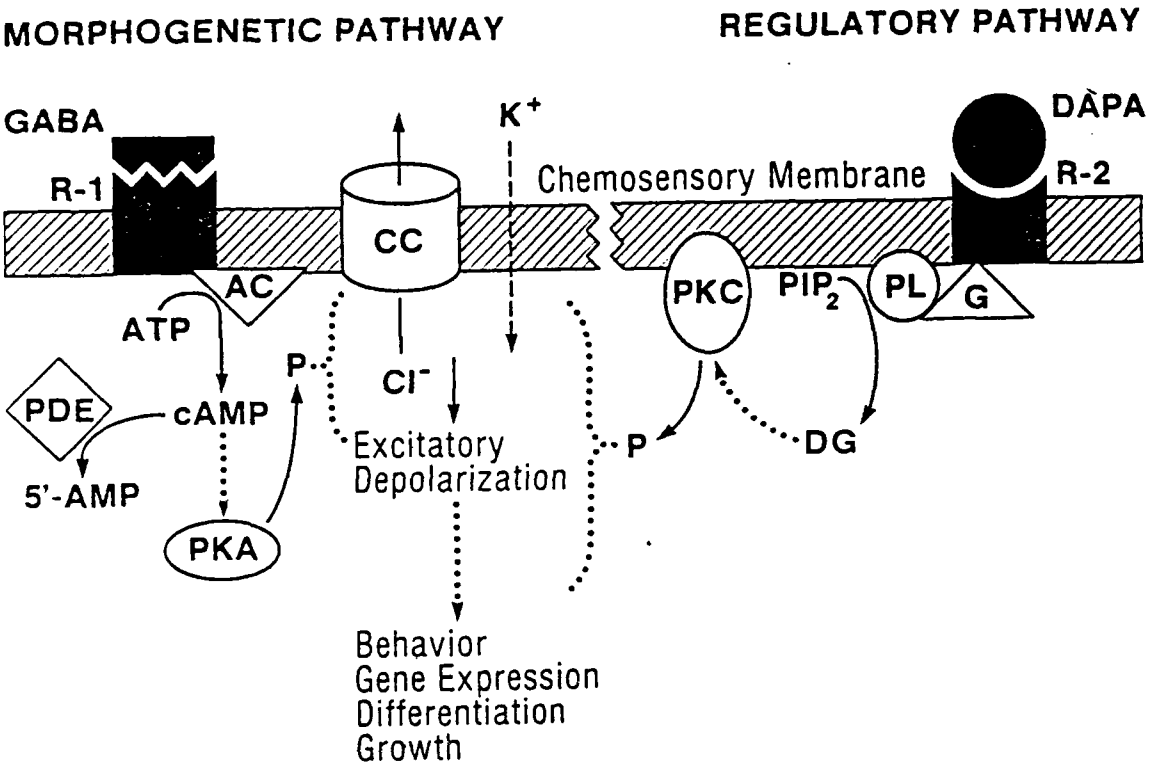
WORK PLAN (for Year 2):

Objective 1: Chemosensory receptors and signal transducers: Our objective is to further characterize the partially purified receptor-transducer complexes *in vitro*; verify their functional integrity; and then use these for the final purification,

resolution, and *in vitro* reconstitution needed to completely determine the molecular mechanisms of their action and regulation. Functional integrity of the Morphogenetic Pathway will be ascertained by measurements *in vitro* of ligand- and receptor-dependent sequential activation of adenyl cyclase, protein kinase A, and chloride ion channel opening, leading to a receptor-dependent efflux of radioactive chloride ion from the re-sealed ciliary membranes. Functional integrity of the diamino acid-dependent Regulatory Pathway will be ascertained by measurements of a ligand- and receptor-dependent and G protein-dependent sequential activation of phospholipase activity, production of diacylglycerol, and the subsequent activation of protein kinase C. Substrates for the protein kinase-mediated phosphorylations in both signal transduction pathways will be characterized by gel electrophoresis, autoradiography, and protein fingerprinting. The molecular site and mechanism of interaction of the Morphogenetic and Regulatory pathways will be identified *in vitro*. We then will attempt the final purification, characterization, and functional reconstitution of the elements involved in the receptor-dependent signal transduction in both pathways, and will unequivocally determine their molecular mechanisms of action and regulation.

*Objective 2*: Mechanism of morphogenetic activation of specific gene expression in the developmentally arrested larva: We will complete the sequence analysis of the cloned *Haliotis* insulin gene now in progress; identify putative transcription-regulating sequences by chromosome-walking, subcloning and sequence analysis; and confirm these identifications by expression of the cloned gene *in vitro*, with detection of the gene product by monoclonal antibody immunoprecipitation.

The cloned gene sequence then will be used as a sensitive probe, in both dot-blot and Northern hybridization analyses, to detect and characterize the expression of the insulin gene following morphogenetic activation of the *Haliotis* larva. Using this assay, both *in vivo* and *in vitro*, we will attempt to identify the mechanisms by which the morphogenetic peptide signal and the larval signal transduction pathway (see section above) activate this gene expression.




The receptors and signal transducers of the Morphogenetic and Regulatory Pathways that control settlement, metamorphosis and gene activation in Haliotis larvae in response to exogonous signals have been partially purified and characterized in vitro. The Haliotis insulin (or IGF) gene, that is activated early in metamorphoisis, has been cloned and partially sequenced.

R-1 and R-2 are external chemosensory receptors; GABA = gamma-aminobutyric acid or GABA-mimetic peptide; DAPA = diaminopropionic acid; AC = adenylate cyclase; PDE = cAMP-phosphodiesterase; PKA = protein kinase A; CC = chloride ion channel; G = G protein; PL = phospholipase; $PIP_2$ = phosphatidylinositol bisphosphate; DG = diacylglycerol; PKC = protein kinase C; arrows = catalyzed reactions and resulting depolarization; dotted lines = activations. [Figure from Baxter and Morse, Proc. Natl. Acad. Sci. 84:1867-1870 (1987).]

PUBLICATIONS AND REPORTS (Year 1):

1. A paper reporting the latest results in our purification of the chemosensory receptors and the demonstration of the functional integrity *in vitro* of the first receptor-transducer link is now in preparation.

2. An invited review paper is now in press in *Oceanus*:

   Morse, D.E. and A.N.C. Morse. (1988). Learning from larvae: Chemical signals and molecular mechanisms controlling reproduction and metamorphosis, with wide applications. *Oceanus*, (in press).

3. The following Abstracts are now in press:

   Morse, D.E., A.N.C. Morse, G. Baxter and R.A. Jensen. (1988). Recent progress in the characterization of chemical signals, larval receptors, signal transducers and amplifiers controlling the settlement and metamorphosis of marine invertebrate larvae. *Amer. Zool.* (in press).

   Morse, D.E. (1988). Morphogens, signal molecules, and other non-toxic bioactive substances that play a role in structuring interactions and distributions in the marine environment. *Proc. Intl. Symp. Marine Bioactive Substances* (in press).

   Morse, D.E. (1988). Chemical signals control site-specific settlement and metamorphosis of planktonic larvae: Characterization of the signals, receptors, transduction and regulatory mechanisms. *J. Chem. Ecol.* (in press).

   Morse, D.E. (1988). Molecular mechanisms controlling larval settlement and metamorphosis: A focus of the molecular marine biology program at the University of California, Santa Barbara. *Proc. First Intl. Symp. Mar. Molec. Biol.* (in press).

GRADUATE STUDENT TRAINING ACTIVITIES:

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|---|---|
| Graduate Student Trainees | -2 |
| Women or Minorities | -0 |
| Non-citizens | -0 |

AWARDS/FELLOWSHIPS:

Visiting Fellow, Smithsonian Institution, Carrie Bow Cay Laboratory (January, 1988).

OTHER HONORS:

Appointed Chairman and Organizer, NSF Taskforce on the Transfer of Molecular Biology and Biotechnology to Ocean Sciences (1987).

Appointed Chairman, Marine Biotechnology Center, University of California at Santa Barbara (1987).

Regents Fellow, Smithsonian Institution, Washington, D.C. (1987-1988).